

Lab-on-a-chip technology

By enabling fluids to be handled in very small volumes, lab-on-a-chip technology permits the integration of several sequential experimental steps into one single automated process.

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One of the everyday steps in life science research is determination of the size, quality and concentration of biomolecules such as DNA, RNA and proteins, and the analysis of fluorescent parameters of cells. Traditional methods in pharmaceutical research – such as gel electrophoresis and capillary electrophoresis for biomolecule analysis, or fluorescence microscopy and flow cytometry for cell analysis – are now complemented by a new analytical technique: lab-on-a-chip technology.

Lab-on-a-chip technology enables the performance of several different experimental tasks in combination with automated data analysis in one process on a single instrumental platform. This new technology has several advantages compared with conventional techniques; these include minimal sample requirement, ease of use, quick analysis times and high reproducibility. In this article, we review the development of lab-on-a-chip technology and present some examples of how it can be applied in pharmaceutical research.

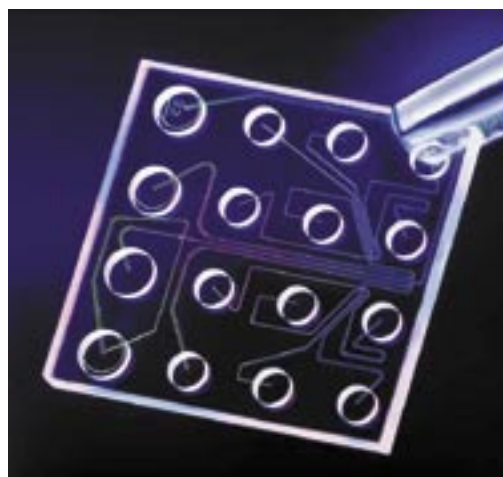
Automated bioanalysis

With the enormous efforts to sequence the human genome and the development of new targeted therapeutics, there is a steadily growing demand for analytical tools which enable much faster and more automated analysis of biomolecules and cells than is currently possible. However, most traditional analytical techniques are still time-consuming and laborious, and require a considerable amount of sample. A recent development, lab-on-a-chip technology (1–6), enables the handling of fluids

in very small volumes within microfluidic channels etched into glass or plastic chips (Figure 1). This technology permits the integration of several sequential experimental steps in an automated manner into one single process.

Many laboratory tasks such as sample handling, electrophoretic separation, staining/de-staining, hydrodynamic cell focusing and detection can now be performed within the confinement of small microfabricated chips. In combination with

Figure 1. Glass chip for protein analysis. Small interconnected channels are etched into glass, including features which allow the analysis of ten protein samples – including sample-handling, electrophoretic separation, staining/de-staining and detection.



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software that automates data analysis and archiving, such a lab-on-a-chip analysis system benefits from minimal manual intervention, low sample consumption, increased speed of analysis, greater data precision, significant cost reduction and minimal exposure to hazardous materials. One of the first commercially available instruments based on lab-on-a-chip technology was developed by Agilent Technologies in collaboration with Caliper Technologies; this was the Agilent 2100 bioanalyser.

Lab-on-a-chip system

The Agilent 2100 bioanalyser consists of a bench-top device (chip reader) that communicates with a PC. The chip reader contains programmable high voltage power supplies, each of which is connected to a platinum electrode. It also contains a pump to generate pressure-driven flow. The bioanalyser can be equipped with an electrode cartridge for molecular assays or a pressure cartridge for cell assays. The glass chip, with loaded samples, is placed in the instrument where it is connected to platinum electrodes or the pressure source by closing the lid. The electrodes enable the instrument to perform multiple electro-kinetic injections and other fluid manipulations from specific sample wells on the chip. Injection and electrophoretic separation of the sample, detection of the fluorescent signal and data analysis are all fully automated.

For molecular assays, each sample is separated in less than two minutes (90 seconds for a DNA sample, for example), so that the complete run of a chip with 10 to 12 samples – including the warm-up phase and calibration of the instrument – is completed within 25–30 minutes.

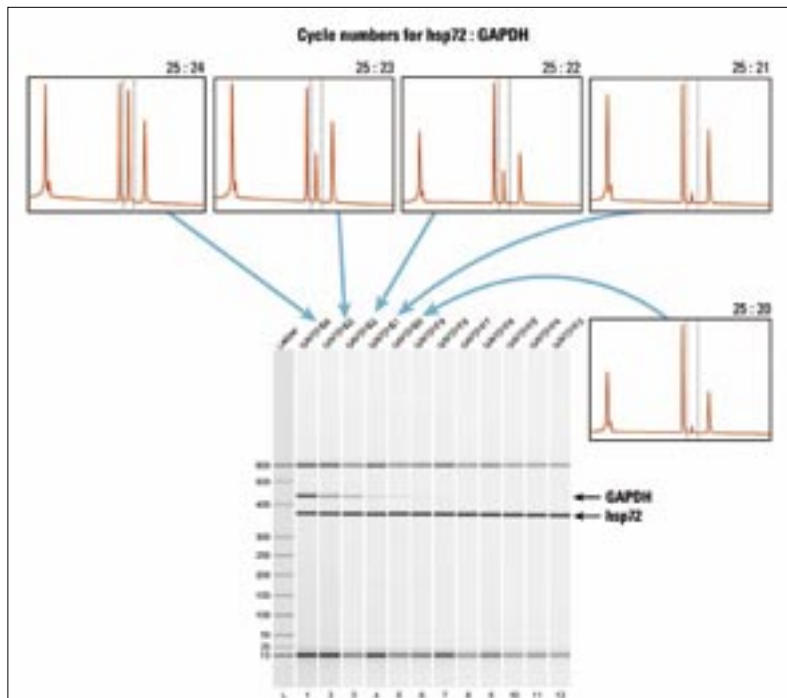
For cell assays, the pressure cartridge controls the movement of cells within the microfluidic channels on the chip by applying a vacuum to one of the wells. Cells are then hydrodynamically focused and pass the fluorescence detectors in single file, thus enabling simple flow cytometric analysis on a chip. Six cell samples can be analysed in less than 30 minutes. The system software includes data collection, presentation and interpretation functions. Data is displayed as a gel-like image and electropherogram for the analysis of biomolecules, or histograms and dot plots for cell assays. In addition, the data is also displayed in tabular format and can be exported to spreadsheet programs for further analysis.

Applications

Lab-on-a-chip technology can perform a variety of laboratory tasks on a chip; these include:

- Sizing and quantification of DNA fragments (for example, PCR products or restriction digests),
- Quality analysis and quantification of total or messenger RNA samples,
- Analysis of recombinant protein expression in cell lysates, optimisation of protein purification procedures and quality control of antibodies, and
- Analysis of intra- and extra-cellular protein expression in cells, determination of the transfection status of a cell population and apoptosis studies.

Figure 2. Gel-like image generated from the electropherograms of the 12 runs displaying a co-amplification of hsp72 (384 bp) and GAPDH (443 bp) for 25 cycles and 24 to 13 cycles respectively. As can be seen, GAPDH could be amplified exponentially whereas hsp72-concentrations remained at a relatively constant level over at least seven cycles.



Specific application examples for lab-on-a-chip technology and its advantages compared with conventional techniques are described in more detail in the following section. All experiments were performed using application-specific LabChip kits and the Agilent 2100 bioanalyser (Agilent Technologies Deutschland GmbH, Waldbronn, Germany).

Sizing and quantification of PCR products

The DNA LabChip kits facilitate rapid separation of double-stranded DNA and deliver higher quality data than classical slab-gel electrophoresis. Quantification in particular is greatly improved compared with gels due to the high sensitivity and large linear dynamic range of the assay, leading to improved results in the area of comparative RT-PCR measurements. The following example shows the application of this technology for semi-quantitative RT-PCR. In order to study the up- or down-regulation of genes, it is useful to co-amplify the gene of interest together with another gene, which does not change its concentration in the investigated cell type (the housekeeping gene). If

both genes are present at different levels, the PCR reaction has to be optimised so that both genes can be detected during exponential amplification.

Heat shock protein (hsp72) and GAPDH were co-amplified at varying cycle numbers (Figure 2). While hsp72 was amplified constantly at 25 cycles, GAPDH cycle numbers varied between 24 and 13. Using GAPDH as an internal standard (housekeeping gene), the up- or down-regulation of hsp72 can be monitored with high precision. This approach represents a simple and cost-effective way to study gene expression via RT-PCR.

Quality control of total RNA The analysis of intact total RNA is shown in Figure 3. The 18S and 28S ribosomal RNA bands are automatically detected by the software and dominate the electropherogram. A lower marker is added to each sample to allow for sample alignment and easy sample comparison. Degradation of total RNA due to RNase contamination results in a shift of the RNA size distribution to smaller fragments and a decrease in signal intensity. The 18S and the 28S RNA bands can no longer be identified with certainty. With progressive degradation, only small fragments are detected and the signal intensity decreases further.

Sizing and analysis of proteins A specific chip design also permits the analysis of up to ten protein samples in the size range from 14–200 kDa within less than 30 minutes with the lab-on-a-chip system (7). In contrast, it would take

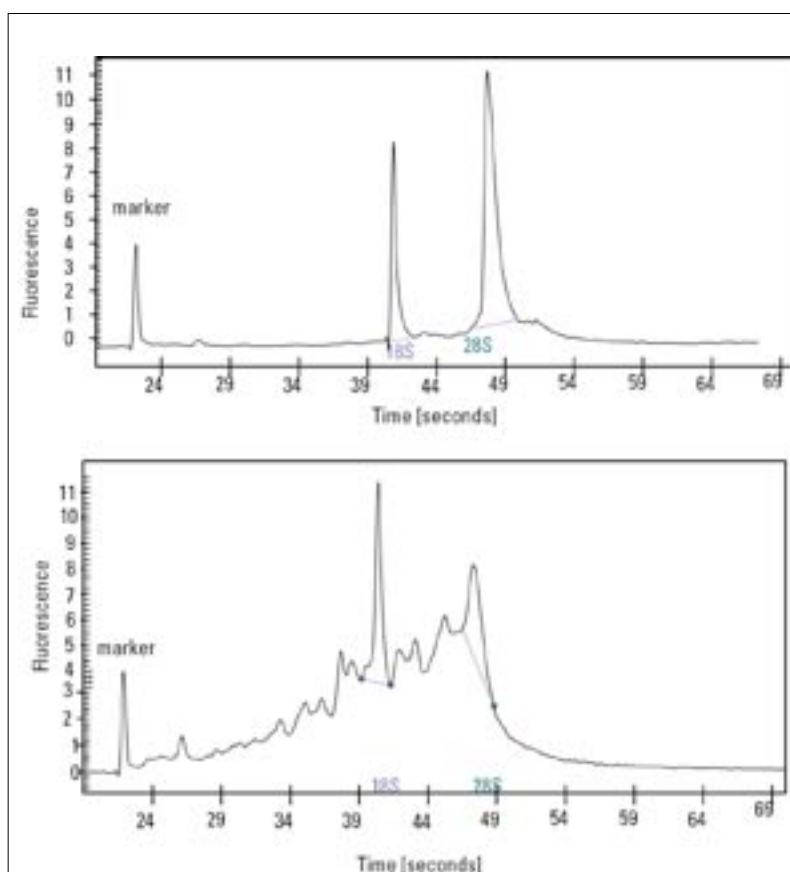


Figure 3. Total RNA was incubated at room temperature with a diluted RNase (2×10^{-6} and 1×10^{-5} mg/ml). The results from the lab-on-a-chip system (RNA 6000 Nano LabChip kit) are displayed as gel-like image (right) and electropherogram (left). The first electropherogram shows intact total RNA, and the second electropherogram shows RNA degradation due to RNase contamination.

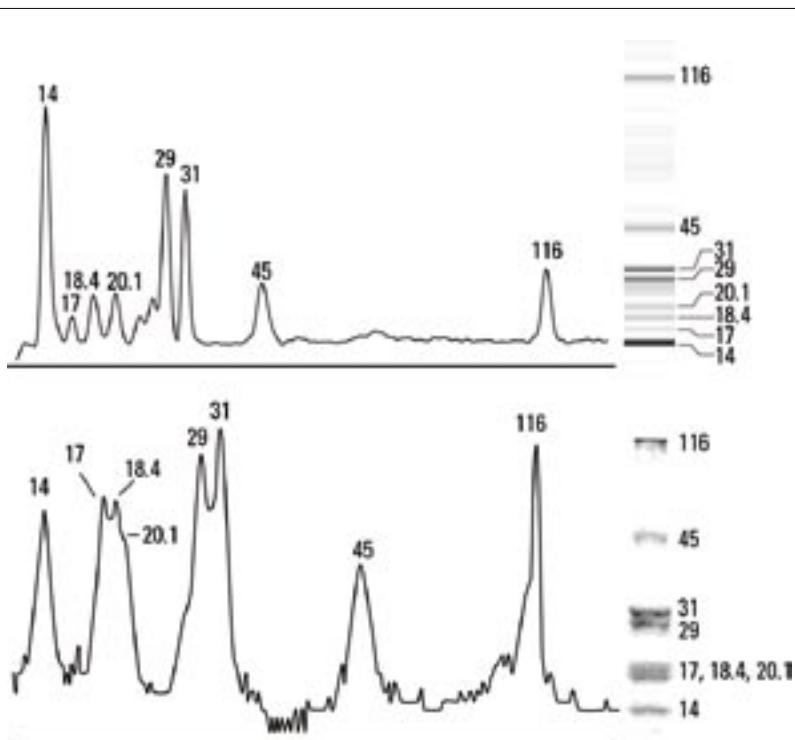


Figure 4. Analysis of a protein mixture with the lab-on-a-chip system in comparison with the analysis of the same protein sample with a 4–20% gradient polyacrylamide gel. The electropherogram together with the gel-like image from the lab-on-a-chip system are shown in the upper panel. The lower panel shows the image from a polyacrylamide gel together with the scan. The molecular weights are indicated in kDa.

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about three hours by SDS-PAGE using pre-cast gels to obtain the same data. These significantly shorter analysis times are possible since lab-on-a-chip technology both integrates and automates many of the manual and time-consuming steps, such as staining and de-staining of proteins. A variety of different

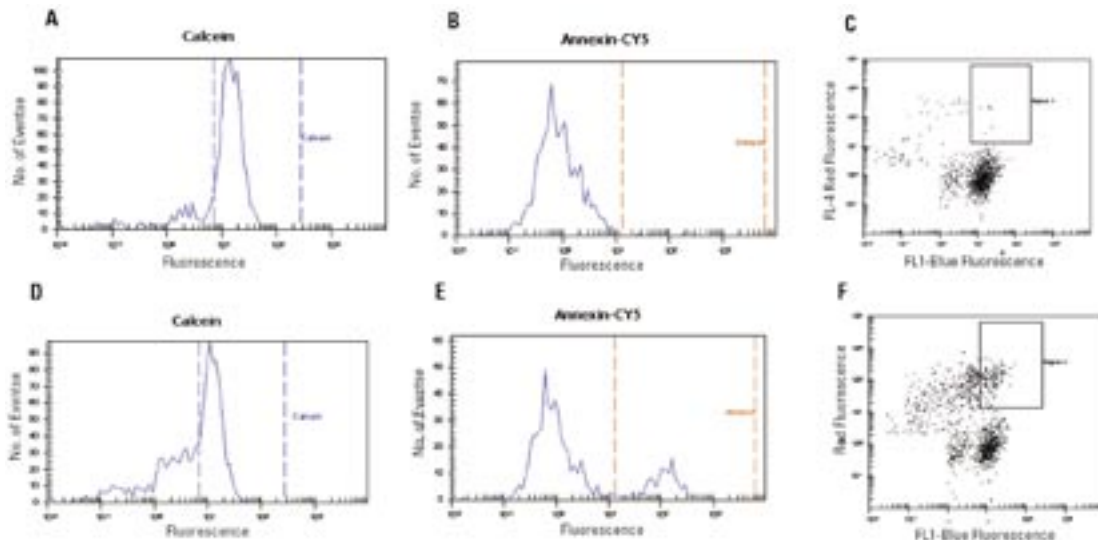
samples can be analysed, such as cell lysates, column fractions, antibodies or other purified proteins. In addition, the sensitivity that can be achieved with the system utilising laser-induced fluorescence detection is comparable with non-colloidal Coomassie gel staining. Figure 4 shows the analysis of a protein mixture with the bioanalyser and a 4–20% gradient polyacrylamide gel. The resolution that can be obtained with the lab-on-a-chip system is comparable with or better than the conventional technique. For example, carbonic anhydrase I and II – which differ by only less than 10% in molecular weight (31 and 29 kDa, respectively) – are baseline separated using the lab-on-a-chip system.

The system has proven to be especially useful for the detection of recombinant protein expression, optimisation of purification procedures and the quality control of antibodies under reducing and non-reducing conditions.

Apoptosis detection by annexin V staining The Agilent 2100 bioanalyser, together with the Cell Fluorescence LabChip kit, can be used to analyse cell fluorescence parameters for a broad range of applications. The following example shows the detection of phosphatidyl serine (PS) exposed on the cell membrane surface as an indication of apoptosis.

Apoptosis was induced in Jurkat cells with camptothecin and harvested after 0 and 16 hours of treatment. Cells were stained with calcein (a live dye) and annexin-V (linked to Cy5 dye) which specifically binds to PS. Figure 5 shows representative histograms and dot plots for an untreated control sample and a sample treated for 16 hours. Cells stained with calcein (Figures 5A and 5D) and annexin-V (Figures 5B and 5E) were measured to define the sub-populations. Cells that show strong annexin-V binding and a strong calcein signal are apoptotic; these apoptotic cells can also be seen in

Figure 5. Induction of apoptosis in Jurkat cells with camptothecin. Jurkat cells were treated with camptothecin, subsequently stained with calcein and annexin V-Cy5 and analysed on the Agilent 2100 bioanalyser (500–1,000 cells counted per sample). A: Calcein. B: Annexin-Cy5 histogram of untreated sample. C: Dot plot of untreated sample. D: Calcein. E: Annexin-Cy5 histogram of 16-hour treated sample. F: Dot plot of 16-hour treated sample.



a dot plot view, as shown in Figure 5C (control) and 5F. By this means, the final result that 44% of all cells of the 16-hour sample were apoptotic is retrieved. The result and the histogram quality are in good agreement with data obtained using a conventional flow cytometer. The main advantages that the compact Agilent 2100 bioanalyser offers for apoptosis cell assays are ease of use, low cell consumption and high reproducibility.

Conclusion

Lab-on-a-chip technology is about to revolutionise the methodology used in pharmaceutical, biochemistry and molecular biology laboratories. Currently, this technology enables rapid, reliable and reproducible sizing and quantification of nucleic acid and protein samples, as well as analysis of fluorescence parameters for single cells. The simple and safe handling of these chips means that several laboratory tasks – which traditionally required analytical gel electrophoresis or flow cytometric analysis – can now be performed on a single platform. By combining these different techniques into one platform, this versatile tool saves on the costs of several other analytical instruments, as well as valuable laboratory space. Furthermore, digitised data enables easy and convenient data-archiving into a database, with subsequent ease of sharing between fellow researchers – both across the bench and around the world.

For the future, microchips will cover an even wider array of analytical applications in many pharmaceutical, biochemistry and molecular biology laboratories, enabling more complex experimental steps – such as sample purification, extraction and biochemical reactions – to be performed on a single chip. In addition, the technology provides the tools to increase experimental throughput significantly.



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References

1. Effenhauser CS, Paulus A, Manz A and Widmer M (1994). *Anal Chem*, 66, 2949–2953.
2. Woolley AT, Mathies RA (1994). *Proc Natl Acad Sci USA*, 91, 11348–11352.
3. Woolley AT, Mathies RA (1995). *Anal. Chem*, 67, 3676–3680.
4. Ogura M., Agata Y, Watanabe K. *et al.* (1998). *Clin. Chem*, 44, 11, 2249–2255.
5. Burns MA, Mastrangelo CH, Sammarco TS, *et al.* (1996). *Proc Natl Acad Sci USA*, 93, 5556–5561.
6. Müller O, Hahnenberger K, Dittmann M (2000). *Electrophoresis*, 21, 1, 128–34.
7. Bousse L, Mouradian S, Minalla A, *et al.* (2001). *Anal. Chem*, 73, 6, 1207–1212.

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